Chapter 5

Circadian Rhythm in Locomotor Activity
5.1 Introduction

Study of circadian rhythms of locomotor activity of any organism provides us with the opportunity to elucidate the relationship between the temporal organization of behaviour and changes in geophysical cycles. Among the crustaceans, the freshwater and marine decapods have been used as experimental models to study circadian organization in invertebrates (Warner, 1977; DeCoursey, 1983; Brown, 1983; Árechiga et al., 1993; Fuentes-Pardo and Hernández-Falcón, 1993). Notably among those are: (1) American horseshoe crab, *Limulus polyphemus* (Chabot et al., 2007, 2004); (2) American freshwater crab, *Pseudothelphusa americana* (Vania et al., 2005); (3) Eastern Pacific fiddler crab, *Uca princeps* Smith (Stillman and Barnwell, 2004); (4) the fiddler crab, *Uca subcylindrica* Stimpson (Thurman and Broghammer, 2001; Thurman, 1998); (5) shore crab, *Carcinus maenas* (Naylor, 1996; Reid and Naylor, 1990); (6) the East African fiddler crabs, *Uca urvillei* and *Uca annulipes* (Lehmann et al., 1974). The common concept that emerges from studies on all these experimental models is that the overt rhythms in locomotor activity are controlled by multioscillatory system. In most of the species of crabs, studied thus far, LD-synchronized locomotor activity rhythms have been observed in addition to circatidal rhythms. They prominently exhibit bimodal activity pattern under LD schedules characterized by occurrence of peaks in activity corresponding to light-on and light-off timings. The locomotor activity rhythm has also been reported to free-run under DD and LL in freshwater crab, *Pseudothelphusa americana* (Miranda-Anaya et al., 2003b) and fiddler crab, *Uca subcylindrica* (Thurman and Broghammer, 2001; Thurman, 1998). Interestingly, the bimodality in the pattern
of locomotor activity has been found to remain unaltered upon transfer from LD to DD, whereas following transfer to LL unimodal pattern in locomotor activity has been conspicuous (Miranda-Anaya et al., 2003b). It has been proposed that the coupling of multiple oscillators that drive locomotor activity in decapods is regulated by light (Thurman and Broghammer, 2001).

There is paucity of information on the circadian organization of locomotor activity rhythm in crabs from Indian subcontinent, excluding a solitary paper on tidal and diurnal rhythms of locomotion in sand crab, *Emerita asiatica* (Chandrashekaran, 1965). The author has explained the elevated level of activity in *E. asiatica* during the high tide in the night as consequences of superimposition of diurnal rhythm of activity on a tidal rhythm.

In the present study, it was examined if the locomotor activity of both male and female Indian freshwater crab, *Barytelphusa cunicularis* is rhythmic under LD 12:12 photo cycles and if this rhythm free runs following their transfer from LD to DD or LL. Further, it was investigated if *B. cunicularis* exhibits bimodal pattern of locomotor activity under LD, DD and LL schedules.
5.2 Materials and Methods

5.2.1 Procurement of freshwater crab and acclimation

Live specimens of freshwater crab, *Barytelphusa cunicularis* were collected from local wetlands and freshwater ponds during rainy season and transferred to animal house. Male and female crabs were segregated and kept in separate stock aquaria containing tap water. The crabs were fed with boiled egg white on alternate day and fresh tap water was replenished a day after each feeding. They were acclimated to the laboratory conditions for 15 days. During the period of acclimation the crabs were exposed to LD 12:12 (Light onset: 06:00) photoperiod and relatively constant room temperature that varied between 22°C and 24°C. The light fraction of the LD had an intensity of 150 lux.

5.2.2 Recording of locomotor activity

Crabs were kept individually in a specially design transparent rectangular aquaria measuring 40 x 10 x 15 cm. The bottom of each aquarium consisted of two parts, such as aquatic zone and terrestrial zone. The former was filled with tap water up to 5 cm height. Each crab was provided with the option to select either of the zones. The crabs had predilection for the aquatic zone. The rhythmic locomotor activity of the crabs were monitored and recorded by using IR sensors placed in the middle of the aquatic zone. The sensor alignment was confirmed through light indicators positioned in the rear part of each sensor. IR beam interruptions caused by the movement of crabs were fed to a 20-channel Angus event recorder (Figure 5.1 and Figure 5.2).
5.2.3 Experimental protocol

Experiment 1: Five LD-acclimated male crabs (average body weight = 58.59 g) were randomly selected and were exposed to DD for 10 days. Thereafter, they were exposed under LD 12:12 (Light onset: 06:00), DD, LD 12:12 (Light onset: 06:00), and LL in a sequential manner. In each schedule they had an experience of 10-12 days. Animal husbandry and maintenance activities were carried out under red dim light during the dark fraction of LD schedules and DD.

Experiment 2: The experimental protocol was identical with that of the experiment 1, but the specimens were randomly chosen female crabs (average body weight: 56.81 g) and that they were exposed under DD, LD, DD, LD, and LL sequentially for 15 days each.

5.2.4 Construction of actogram and digitization of data

Actogram was constructed day wise by placing locomotor activity record of each day, one below the other. Double plotting of activity was executed for the better visualization of rhythmic patterns. The qualitative data on the actogram were digitized, using the technique adopted by Thurman and Broghammer (2001). Each hour on the actogram was divided into six 10-minute bins and scored for activity. To represent the sum of the activities for the entire hour integers between 1 and 7 were used. The former digit indicated that the crab was dormant during the entire hour and the latter indicated that the crab was active at least once during all six 10-minute bins. The process of digitization was completed for each day and each time series (Figure 5.3)
5.2.5 Statistical Analysis of Data

Data were analyzed for documenting a circadian rhythm in locomotor activity (τ = 24 h) with the help of Cosinor rhythmometry (Nelson et al., 1979). A rhythm was characterized by estimating three parameters, such as the Mesor (M, rhythm-adjusted mean), the amplitude (A, half of the difference between the highest and the lowest value of the rhythmic function) and the acrophase (Ø, timing of the highest value of the rhythmic function). A power spectrum method was also employed for detecting prominent period (τ) in individual time series for locomotor activity (De Prins et al., 1986). Other conventional analysis, viz., descriptive statistics, ANOVA, and Duncan's multiple-range test, were performed whenever needed.

5.3 Results

5.3.1 Locomotor activity rhythm

Both male and female crabs were more active during the onset of dark phase. The daily records of locomotor activity of one male and one female, chosen as representatives, are depicted in Figure 5.4 and Figure 5.5, respectively. A bout of increased activity was also noticeable corresponding to the timings of light onset, while crabs were under LD 12:12. This bimodality in locomotor activity of crabs disappeared, when they were subjected to DD or LL, irrespective of gender (Figure 5.4 and Figure 5.5). The observed bimodality was more distinct and
precise in case of female crab (Figure 5.5). However, in general the rhythm
detection ratio was of lower order in the group of female crabs (Table 5.1).

5.3.2 Rhythm-adjusted mean, Mesor

The male crabs were always more active as compared with the females,
irrespective of the photo regimens. This was gauged from the values of circadian
Mesor (Table 5.1). However, the Mesor in the group females was not statistically
significant from that of the males when they were exposed under LL and LD
12:12 following DD. In males Mesor under DD was always higher as compared
with that when they were exposed under LL. This difference was not marked in
case of female crabs (Table 5.1).

5.3.3 Amplitude

The amplitude of locomotor activity remained invariant irrespective of gender and
photo regimens. Although differences among values of amplitudes were not
statistically significant in respect of gender, it was always lower in females
irrespective of photo regimens, namely LD 12:12, DD and LL (Table 5.1).

5.3.4 Peak

The timings of peak in locomotor activity did not alter statistically significantly as
function of gender (Table 5.1), but it occurred earlier in males under LD 12:12 as
compared with that when there were under DD and LL. In the former the advance
was statistically significant (Table 5.1). In females also the timings of peak in
locomotor activity occurred earlier while they were under LD 12:12 as compared with that under DD and LL. In females the said phase advances under LD 12:12 was not statistically significant (Table 5.1).

5.3.5 Period

Prominent periods gauged from spectral analysis are shown in Figure 5.6 and Figure 5.7. They depict periodograms of two representative male and female crabs, respectively. In general under LD 12:12, a bimodal pattern in locomotor activity was evident in both male and female crabs. This distinction was more prominent in case of female crabs as evident from the actogram (Figure 5.8). The locomotor activity free-ran under constant conditions, such as DD and LL. The Figure 5.8 illustrates the mean period length in groups of male and female crabs under LD 12:12, DD and LL. The period length was shorter under LL as compared with that under DD, irrespective of gender, although the difference was not statistically significant (Figure 5.8).
Chapter 5 – Circadian Rhythm in Locomotor Activity

Figure 5.1 Experimental setup

Figure 5.2 Experimental setup
Figure 5.3 Digitization of Actogram
Figure 5.4 Double-plotted actogram of a representative male Barytelphusa cunicularis (BcM # 05) under DD following LD 12:12, DD, LD 12:12 and LL.

Each line represents one 48-h period and successive days are shown from top to bottom.
Figure 5.5 Double-plotted actogram of a representative female *Barytelphusa cunicularis* (BcF # 05) under DD following LD 12:12, DD, LD 12:12 and LL.

Each line represents one 48-h period and successive days are shown from top to bottom.
Chapter 5 – Circadian Rhythm in Locomotor Activity

Figure 5.6 Periodogram of the locomotor activity rhythm of a representative male *Barytelphusa cunicularis* (BcM # 05) under DD following LD 12:12, DD, LD 12:12 and LL.
Figure 5.7 Periodogram of the locomotor activity rhythm of a representative female *Barytelphusa cunicularis* (BCF # 05) under DD following LD 12:12, DD, LD 12:12 and LL.
Figure 5.8 Average prominent periods of locomotor activity rhythm in the groups of male and female crabs exposed to different light schedules.
Table 5.1: Cosinor summary of the characteristics of locomotor activity rhythm in freshwater crab, *Barytelphusa cunicularis*.

<table>
<thead>
<tr>
<th>Schedule</th>
<th>Male Rhythm-adjusted average of the best-fitting cosine function ± 1 SE</th>
<th>Female Rhythm-adjusted average of the best-fitting cosine function ± 1 SE</th>
<th>A</th>
<th>M ± 1 SE</th>
<th>M ± 1 SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>DD1</td>
<td>2.81 ± 0.14'·b 0.45 ± 0.10' 19.92 ± 0.92'·b 18.71 ± 1.0'·b</td>
<td>2.25 ± 0.09'·a·b·' 0.48 ± 0.13' 10.94 ± 3.9''d</td>
<td>240</td>
<td>360 0.8 0.6 5.80 ± 1.89' 10.94 ± 3.9''d</td>
<td>264</td>
</tr>
<tr>
<td>DD2</td>
<td>2.85 ± 0.12' 0.32 ± 0.07' 13.98 ± 1.47' 13.21 ± 3.3a,b,c,d</td>
<td>2.05 ± 0.09'·a·b·' 0.29 ± 0.06' 10.53 ± 2.86''d</td>
<td>264</td>
<td>360 0.8 0.6 5.80 ± 1.89' 10.94 ± 3.9''d</td>
<td>264</td>
</tr>
<tr>
<td>LD1</td>
<td>2.43 ± 0.17'·b·o 0.45 ± 0.10' 10.53 ± 2.86''d 13.21 ± 3.3a,b,c,d</td>
<td>2.32 ± 0.13'·b·o·l 0.44 ± 0.08' 10.53 ± 2.86''d 13.21 ± 3.3a,b,c,d</td>
<td>288</td>
<td>360 0.4 0.4 10.53 ± 2.86''d 13.21 ± 3.3a,b,c,d</td>
<td>288</td>
</tr>
<tr>
<td>LD2</td>
<td>2.18 ± 0.12'·b·o·l 0.25 ± 0.05' 12.23 ± 4.5''f 19.72 ± 4.5''f</td>
<td>1.92 ± 0.17d 0.33 ± 0.08' 12.23 ± 4.5''f 19.72 ± 4.5''f</td>
<td>240</td>
<td>360 0.6 0.6 12.23 ± 4.5''f 19.72 ± 4.5''f</td>
<td>240</td>
</tr>
</tbody>
</table>

Rhythm-adjusted average of the best-fitting cosine function ± 1 standard error; A, half the difference between the maximum and the minimum of the best-fitting cosine function ± 1 SE; 0, time in hour of the maximum in the best-fitting cosine function ± 1 SE.
5.4 Discussion

*Barytelphusa cunicularis* is a brachyuran (family: Potamonide) freshwater crab and is widely distributed in the tropical and sub-tropical regions of the Indian subcontinent. Although it provides biologists with an excellent opportunity to study the characteristics of circadian rhythm of its locomotor activity and to compare its chronophysiology with that of its marine counterparts, there is not even a single paper on the study of biological rhythm in this species.

Most of the marine crabs exhibit both circadian and circatidal rhythms. A true circatidal rhythm (c. 12.4 h) in locomotor activity of shore crab, *Carcinus maenas* has been well documented (Naylor, 1996). *C. maenas* also exhibits circadian rhythm (c. 24 h) in locomotor activity. The resultant wave form in locomotor activity of *C. maenas* has been ascribed to interactions between the circadian and circatidal oscillations (Naylor, 1996). Similar phenomenon has been reported long back in Indian sand crab, *Emerita asiatica* (Chandrashekaran, 1965). There are many other studies that document such interaction in marine crabs (Reid and Naylor, 1990; Thurman, 1989; Thurman and Broghammer, 2001; Stillman and Barnwell, 2004). Multiple oscillators' hypothesis has also been proposed for the rhythm in locomotor activity of freshwater crab, *Pseudothelphusa americana* (Miranda-Anaya et al., 2003b).

In the present study, a conspicuous bimodality in locomotor activity rhythm of *Barytelphusa cunicularis*, irrespective of gender, is observed under LD 12:12. However, this bimodality, as gauged from the actograms, was much more distinct
in case of female crabs. This phenomenon seems to be of common occurrence among crabs, both freshwater and marine species (Chabot et al., 2007; Vania et al., 2005; Miranda-Anaya, 2004; Miranda-Anaya et al., 2003b; Reid and Naylor, 1990). In *B. cunicularis* activity pattern entrains to LD with two distinct bouts; one coincides with the onset of darkness and the other one with the onset of light. Present results support earlier findings for *Uca subcylindrica* (Thurman and Broghammer, 2001).

The level of overall activity is more in male *B. cunicularis* as compared with its female counterparts under all light schedules, except LL. However, the level of overall activity under the latter was also more in male crabs, although it was not statistically significant. In most of the earlier studies the gender of the test model has not been specified; therefore it would be difficult to compare the present findings in respect of level of activity. It is generalized that in *B. cunicularis* males are always more active than the females, irrespective of the light schedules to which they are exposed. However, in respect of circadian amplitude and peak there are no significant differences, when the locomotor activity rhythms of male and female crabs are compared. The circadian amplitude remains invariant irrespective of the light schedules. Nonetheless, circadian amplitude was always higher in males as compared with the females, although the difference is not statistically significant. The lower values of rhythm detection ratio in females under different photo regimes complement the above observation. Unfortunately no peer studies are available mentioning about the nature of variation in circadian amplitude as function of either light schedules or gender.
The peak in locomotor activity rhythm always advances when the crabs are exposed to LD 12:12 schedules as compared to their exposure under either DD or LL. This could be imputed to free-running nature of the locomotor activity rhythm in both male and female crabs. The period of the locomotor activity rhythm in crabs, both males and females, shortens, when they are exposed to LD 12:12 light schedule. Under LD, there is no significant difference between males and females as regards lengths of period are concerned. Under LD 12:12, the average of period length is about 12 h, irrespective of gender. This further consolidates bimodality as a prominent feature in *B. cunicularis* under conditions of LD entrainment and corroborates earlier findings on bimodality in locomotor activity rhythm in different species of crabs (Chabot et al., 2007; Vania et al., 2005; Miranda-Anaya, 2004; Miranda-Anaya et al., 2003b; Reid and Naylor, 1990). The rhythm of locomotor activity turns unimodal and free runs under constant conditions, such as DD and LL. It is also noteworthy to mention here that freeruns of locomotor activity rhythm in *B. cunicularis* are not very much conspicuous. In *P. americana* unimodal free-running rhythm of locomotor activity has been reported only under LL, but not DD (Miranda-Anaya et al., 2003b). Therefore, the present results support the earlier findings of Miranda-Anaya et al. (2003b) only partly.

It is concluded that the locomotor activity rhythm of both male and female *B. cunicularis* has the ability to entrain to LD 12:12 cycles and that the rhythm free runs under DD or LL. However, in general the bimodality in locomotor activity rhythm under LD 12:12 disappears, when they are exposed under either DD or LL. This phenomenon appears to be independent of gender. It seems that circadian component in locomotor activity in *B. cunicularis* is strong, irrespective of gender.